CHAPTER 4

Determination of the Xer phenotype of ArgRWT- and ArgRNV- biotinylated peptide fusion protein

CHAPTER 4

Determination of the Xer phenotype of ArgRWT - and ArgRNV - biotinylated peptide fusion protein

4.1 Introduction

This chapter describes the intracellular analysis of the biotinylated peptide fusion derivatives of ArgRWT and ArgRNV activity in Xer site-specific recombination. *In vivo* Xer site-specific recombination assays were performed using *cer* reporter plasmids (Colloms *et al.*, 1990; Chen *et al.*, 1997). Analysis of *cer*-mediated recombination of ArgRWT and ArgRNV fusion proteins is based on their ability to resolve *cer* reporter plasmids into resolution products. Resolution of the reporter plasmid is similar to the resolution of multimeric plasmid into monomeric plasmid (Figure 4.1).

The reporter plasmids allow the assay of *cer*-mediated recombination by phenotypic and electrophoretic techniques. These reporter plasmids contain two directly repeated *cer* recombination sites flanking a reporter gene such as antibiotic resistance or *lacZ*. Recombination between the two *cer* sites in a wild-type host (Xer^+ strain, eg. DS941) will delete the reporter gene and yield two smaller size resolution products; one carries a single *cer* site but lacks a replication origin and hence is rapidly lost from bacterial culture and an autonomous replication plasmid that carries the other *cer* site and a reporter gene (Figures 4.2 and 4.5). In this chapter, two reporter plasmids were used, pCS202 (Cm^rTc^r) and pSH10 (Km^r). The properties of these plasmids are described below.

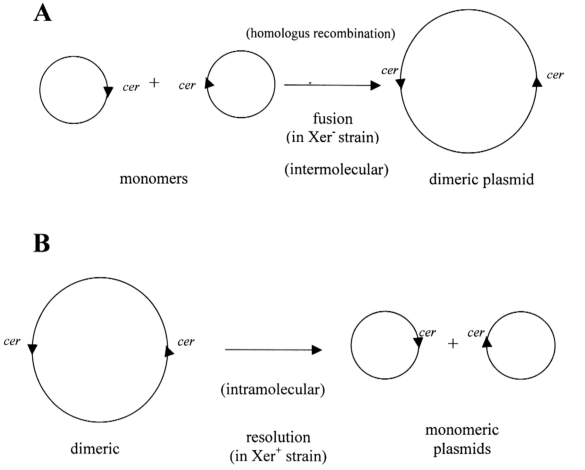

Figure 4.1. Diagrammatic representation of intermolecular and intramolecular Xer recombination. Panel A: Homologous recombination between two monomeric plasmids (fusion), each containing a single *cer* site, gives a dimeric plasmid with two symmetrically located *cer* sites. Such recombination occurred in Xer⁻ strain. **Panel B:** Xer site-specific recombination resolve dimer to monomers (resolution), each with single *cer* site. Such recombination occurred in Xer⁺ strain.

4.2 *In vivo cer*-mediated recombination using pCS202 as reporter plasmid

To determine whether ArgRWT-biotinylated peptide fusion protein is able to support Xer site-specific recombination, the reporter plasmid pCS202 was introduced into DS956 cells containing pKAR100 referred to as DS956 (pKAR100). Plasmid pCS202 is a Cm^rTc^r lambda *dv*-based reporter plasmid containing two directly repeated *cer* sites flanking a copy of the Tc^r gene (Colloms *et al.*, 1990). In a wild-type host (DS941), the Tc^r gene is deleted on *cer*-mediated recombination, yielding two smaller size plasmids (Figure 4.2), a 2.3 kb non-replicative plasmid which lacks a replication origin, that will be lost rapidly from bacterial cultures and a 5.2 kb replicative plasmid (pCS203) carrying the Cm^r gene.

Resolution of pCS202 in DS956 (pKAR100) was analyzed both phenotypically (by indirectly scoring the deletion of a functional Tc^r gene from pCS202) and electrophoretically. DS941 (Xer^+ strain) and DS956 (*argR*$^-$) were also separately transformed with pCS202. Phenotypic analysis of cells grown on selective media showed that in wild-type strain (DS941), the reporter plasmid is totally resolved and that cells can only grow on LB + Cm because the Tc^r gene was deleted from pCS202 during *cer*-mediated recombination (Table 4.1). In DS956 (which lacks ArgR) almost all of the reporter plasmid (45 of 46) were not resolved because they showed growth on LB containing Cm and Tc (Table 4.1). DS956 cells expressing ArgRWT-biotinyated peptide fusion protein showed incomplete *cer*-mediated recombinations of the reporter plasmid pCS202 (13 out of 17, Table 4.1). Electrophoretic analysis of plasmid DNAs from the cells

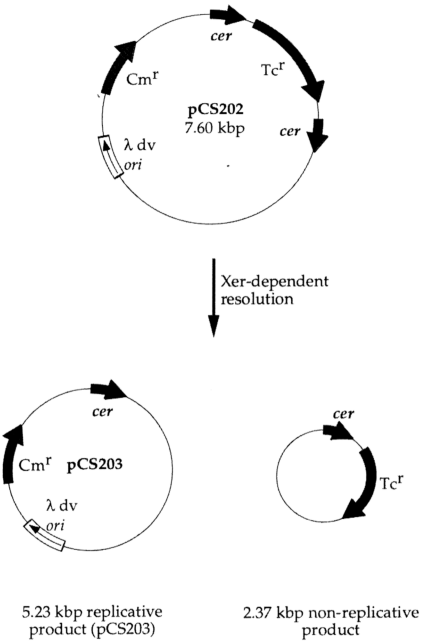

Figure 4.2. Diagrammatic representation of *cer*-mediated recombination of the reporter plasmid pCS202. Plasmid pCS202 encodes resistance to Cm and Tc when maintained in a Xer$^-$ strain (*argR*$^-$ or *pepA*$^-$ or *xerC*$^-$ or *xerD*$^-$). Recombination between the directly repeated *cer* sites on pCS202 in wild-type Xer$^+$ strain yield a 5.2 kb and 2.4 kb circular products. The 5.2 kb replicative plasmid is designated pCS203.

Table 4.1. Phenotypic analysis of *cer*-mediated recombination by ArgRWT-biotinylated peptide fusion protein (expressed from pKAR100), using pCS202 ($Cm^r Tc^r$) as the reporter plasmid.

No.	*E. coli* strain	Number of patched colonies	Number of colonies that grow on LB plates containing antibiotic				Proportion of reporter plasmid resolved (percentage)
			Cm	CmTc	CmAp	CmTcAp	
1	DS956 (without plasmid)	46	46	45	-	-	1 of 46 (2%)
2	DS941 (without plasmid)	16	16	0	-	-	16 of 16 (100%)
3	DS956 (pKAR100, Ap^r)	71	-	-	17	4	13 of 17 (76%)

Note: The deletion of Tc^r marker of pCS202 (Cm^rTc^r) indicating that plasmid pCS202 was resolved to produce pCS203 (Cm^rTc^s). (See subsection 4.3 and Table 4.3).

containing pKAR100 showed very little resolution of the reporter plasmid pCS202 (Figure 4.3, see also Figure 4.6).

The *in vivo* recombination assay was also performed to determine whether ArgRNV-biotinylated peptide fusion protein is able to support Xer site-specific recombination. The reporter plasmid pCS202 was introduced into DS956 (pKAR200), DS941, DS956, and DS956 (pAM401). Both phenotypic (Table 4.2) and electrophoretic analysis (Figure 4.4) showed that ArgRNV-biotinylated peptide fusion protein is proficient in *cer*-mediated site-specific recombination. Plasmid pCS202 showed complete resolution to pCS203 in DS956 cells expressing ArgRNV-biotinylated peptide fusion protein.

4.3 *In vivo cer*-mediated recombination using pSH10 as reporter plasmid

The *in vivo* recombination assay using the reporter plasmid pSH10 was performed to confirm whether ArgRWT and ArgRNV-biotinylated peptide fusion protein support Xer site-specific recombination. Plasmid pSH10 is a reporter plasmid containing *cer-lacZ-cer* 'cartridge' and Km^r gene that deletes *lacZ* gene on *cer*-mediated recombination to generate plasmid pSH11 (Figure 4.5; Chen *et al.*, 1997). Cells harbouring pSH10 are blue in colour because of the expression of the *lacZ* gene while cells harbouring pSH11 are white in colour because it does not contain the *lacZ* gene.

Reporter plasmid pSH10 was separately introduced into DS956 (pKAR100) and DS956 (pKAR200). DS941 (Xer^+) and DS956 (Xer^-), as well as

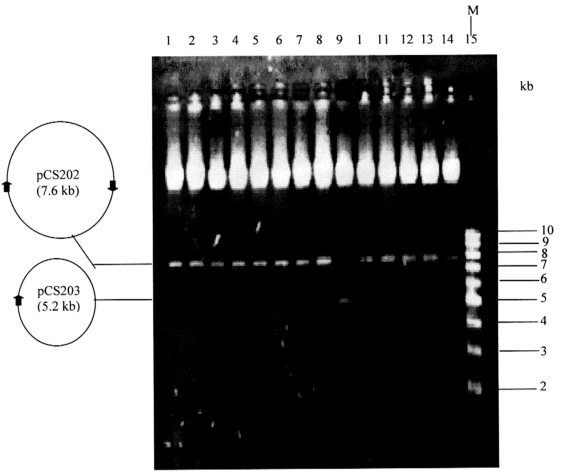

Figure 4.3. *In vivo cer*-mediated recombination by ArgRWT-biotinylated peptide fusion protein (expressed from pKAR100). DS956 cells harbouring pKAR100 (expressing ArgRWT-biotinylated peptide fusion protein) was transformed with a 2-*cer* reporter plasmid, pCS202 (Cm^rTc^r). Individual transformants were patched to CmAp plates, grown, and plasmid DNAs were analyzed by electrophoresis on a 0.8% (w/v) agarose gel after lysis of cells in SCF buffer. Only lane 9 showed the presence of the resolution product (pCS203). pKAR100 was not detected. Lane 15 (M): the supercoiled DNA size marker (the marker sizes are indicated on the right).

Table 4.2. Phenotypic analysis of *cer*-mediated recombination by ArgRNV-biotinylated peptide fusion protein (expressed from pKAR200), using pCS202 (Cm^r Tc^r) as the reporter plasmid.

No.	*E. coli* strain	Number of patched colonies	Number of colonies that grow on LB plates containing antibiotic				Proportion of reporter plasmid resolved (percentage)
			Cm	CmTc	CmAp	CmTcAp	
1	DS956 (without plasmid)	46	46	45	-	-	1 of 46 (2%)
2	DS941 (without plasmid)	16	16	0	-	-	16 of 16 (100%)
3	DS956 (pKAR200, Ap^r)	100	-	-	33	0	33 of 33 (100%)
4	DS956 (pAM401, Ap^r)	63	-	-	63	0	63 of 63 (100%)

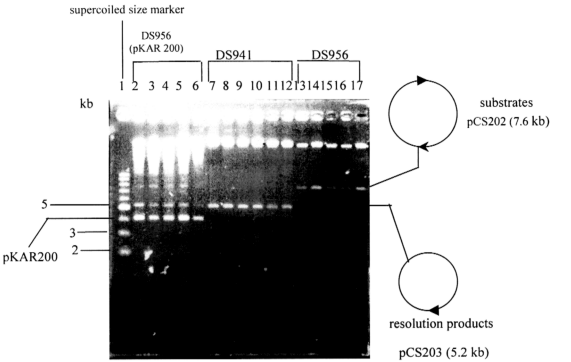

Figure 4.4. *In vivo cer*-mediated recombination by ArgRNV-biotinylated peptide fusion protein (expressed from pKAR200). DS956 harbouring pKAR200 (expressing ArgRNV-biotinylated peptide fusion protein) was transformed with a 2-*cer* reporter plasmid, pCS202 (Cm^rTc^r). Lanes 2-6: *cer*-mediated recombination by ArgRNV-biotinylated peptide fusion protein is shown by the deletion of Tc^r marker from pCS202 (substrates) to produce pCS203 (single *cer*, Cm^r, resolution products). DS941 (without plasmid) as well as DS956 (without plasmid) were also transformed with pCS202. Electrophoretic analysis showed that reporter plasmid, pCS202 was completely resolved in DS941 (Xer^+ strain, lanes 7-12), but pCS202 was not resolved in DS956 (Xer^- strain, lanes 13-17). Lane 1: 2-10 kb supercoiled markers. The sizes are indicated on the left.

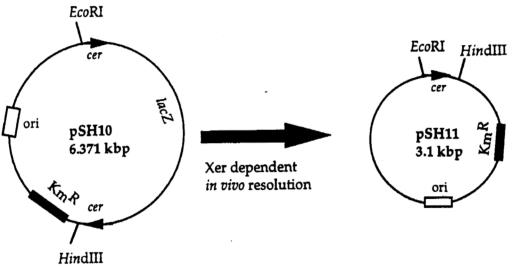

Figure 4.5. Diagrammatic representation of *cer*-mediated recombination of the reporter plasmid pSH10. Xer-dependent resolution of pSH10 deletes the *lacZ* gene to generate pSH11. pSH10 carries *cer-lacZ-cer* 'cartridge' and is resistance to kanamycin. pSH10 is resolved in Xer^+ strains (eg. DS941) to yield pSH11 (3.1 kb; encoding resistance to kanamycin).

DS956 (pAM204) and DS956 (pAM401) were also transformed with pSH10. Phenotypic analysis was performed to determine the resolution of the reporter plasmid pSH10 in DS956 (pKAR100) and DS956 (pKAR200), respectively. The results (Table 4.3) showed that DS956 (pKAR100; expressing ArgRWT-biotinylated peptide fusion protein) showed very little resolution of plasmid pSH10. In contrast, pSH10 was completely resolved in DS956 (pKAR200; expressing ArgRNV-biotinylated peptide fusion protein; Table 4.4).

Electrophoretic analysis showed that the reporter plasmid pSH10 was incompletely resolved into the resolution product pSH11 in DS956 (pKAR100), where very little resolution product was obtained (Figure 4.6). pSH10 showed better resolution in DS956 (pKAR200; expressing ArgRNV-biotinylated peptide fusion protein; Figure 4.7). However traces of pSH10 can be seen in several lanes (Figure 4.7, lanes 1-4), indicating that the resolution is not 100% complete.

This results again indicated that ArgRWT-biotinylated peptide fusion protein poorly supports Xer recombination at *cer*. In contrast, ArgRNV-biotinylated peptide fusion protein support Xer site-specific recombination at *cer* on the reporter plasmid.

Table 4.3. Phenotypic analysis of *cer*-mediated recombination by ArgRWT-biotinylated peptide fusion protein (expressed from pKAR100), using pSH10 as the reporter plasmid.

No.	*E. coli* strain	Number of patched colonies	Number of colonies that grow on LB plates containing antibiotic				Proportion of reporter plasmid resolved (percentage)
			Km (white-colonies)	Km + X-gal (blue colonies)	KmAp (white-colonies)	KmAp + X-gal (blue colonies)	
1	DS956 (without plasmid)	45	45	45	-	-	0 of 45 (0%)
2	DS941 (without plasmid)	45	45	0	-	-	45 of 45 (100%)
3	DS956 (pKAR100, Ap^r)	48	-	-	12	3	9 of 12 (75%)
4	DS956 (pAM204, Ap^R Tc^R)	45	-	-	45	0	45 of 45 (100)

All media were added with IPTG. Final concentration of antibiotics, IPTG, and X-gal were as described in Materials and Methods.

Table 4.4. Phenotypic analysis of *cer*-mediated recombination by ArgRNV-biotinylated peptide fusion protein (expressed from pKAR200), using pSH10 as the reporter plasmid.

No.	*E. coli* strain	Number of patched colonies	Number of colonies that grow on LB plates containing antibiotic				Proportion of reporter plasmid resolved (percentage)
			Km (white-colonies)	Km + X-gal (blue colonies)	KmAp (white-colonies)	KmAp + X-gal (blue colonies)	
1	DS956 (without plasmid)	45	45	45	-	-	0 of 45 (0%)
2	DS941 (without plasmid)	45	45	0	-	-	45 of 45 (100%)
3	DS956 (pKAR200, Ap^R)	45	-	-	40	0	40 of 40 (100%)
4	DS956 (pAM401, Ap^R)	45	-	-	45	0	45 of 45 (100%)

All media were added with IPTG. Final concentration of antibiotics, IPTG, and X-gal were as described in Materials and Methods.

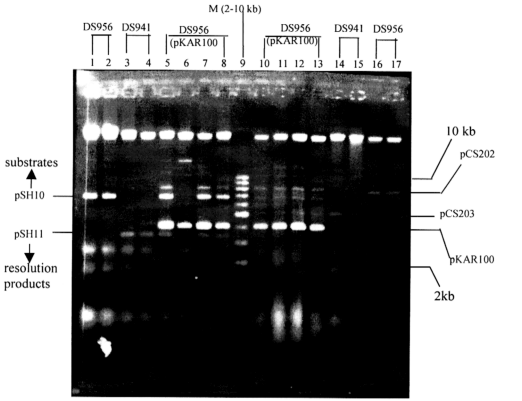

Figure 4.6. ***In vivo cer*-mediated recombination by ArgRWT-biotinylated peptide fusion protein (expressed from pKAR100).** DS956 harbouring pKAR100 (expressing ArgRWT-biotinylated peptide fusion protein) was transformed with a reporter plasmid pSH10 (containing *cer-lacZ-cer* 'cartridge' and Km^r). Electrophoretic analysis showed that pSH10 was incompletely resolved into pSH11 by ArgRWT-biotinylated peptide fusion protein (lanes 5-8). Plasmid pCS202 was also transformed into DS956 (pKAR100) as comparison, and the result once again showed that pCS202 was incompletely resolved into pCS203 (lanes 10-13). DS956 and DS941 were also transformed with pSH10 (lanes 1-2 and lanes 3-4, respectively). DS956 and DS941 were also transformed with pCS202 (lanes 14-15 and lanes 16-17, respectively). Lane 9: 2-10 kb supercoiled size markers.

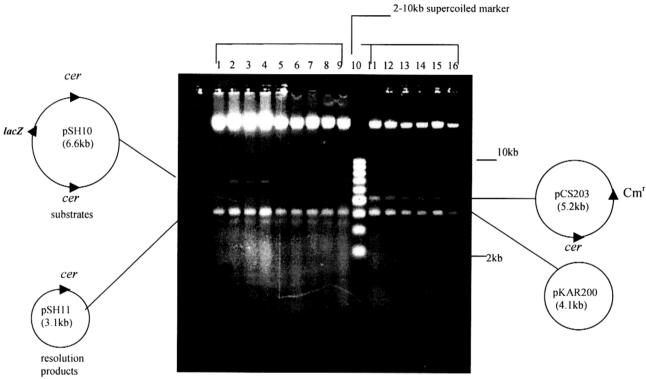

Figure 4.7. *In vivo cer*-mediated recombination by ArgRNV-biotinylated peptide fusion protein (expressed from pKAR200). DS956 harbouring pKAR200 (expressing ArgRNV-biotinylated peptide fusion protein) was transformed with the reporter plasmid pSH10 (containing *cer-lacZ-cer* 'cartridge' and Km^r, lanes 1-9). Electrophoretic analysis showed that pSH10 was resolved incompletely to produce pSH11 (lanes 1-4). Note that pSH10 still remained in bacterial cells. However pCS202 was completely resolved to produce pCS203 (lanes 11-16; see also Figure 4.6). Lane 10: 2-10 kb supercoiled DNA markers (the marker sizes are indicated on the right).